REMARKS

Claims 1, 3, 4, 33, 39 and 84-90 were pending in the instant application. Claims 2, 5-18, 20, 23-26, 28-32, 37-38 and 64-83 were previously canceled without prejudice as directed to a non-elected invention. Claims 19, 21-22, 27, 34-36, 40-63 and 91-108 are withdrawn from consideration as drawn to a non-elected invention. Claims 40-63 (Group II) are related to claims 1-39 and 67-68 (Group I) as product and process of use. It is the Applicant's understanding that, once the pending product claims are found allowable, any non-elected process claims (Group II, claims 40-63) will be rejoined and examined if such process claims include all of the limitations of the elected product claims (MPEP §821.04).

Claim 86 has been amended. Accordingly, claims 1, 3-4, 33, 39 and 84-90 will be pending after entry of the instant amendment. Support for the claim amendments can be found throughout the claims and specification as originally filed. No new matter has been added. In particular, support for the phrase "wherein the antisense strand comprises at least one 2'deoxy adenosine or 2'-deoxy guanosine" can be found at least in Example 10, Figure 10A, Example 13, Figure 19 and Figures 13A, B and C.

The foregoing claim amendments should in no way be construed as an acquiescence to any of the Examiner's rejections and were made solely to expedite prosecution of the present application. Applicant reserves the right to prosecute the claims as originally filed in this or a continuing application.

Acknowledgement of the Withdrawal of Previous Rejections

Applicants gratefully acknowledge the withdrawal of the previous rejection of claim 86 under 35 U.S.C. § 112, second paragraph, as being indefinite.

Rejection of Claims 1, 3-4, 33, 39 and 84-90 under 35 USC § 103(a)

The Examiner has maintained the rejection of claims 1, 3-4, 33, 39 and 84-90 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* (WO 22/44321) in view of Eckstein *et al.*

(US 5,672,695) and Parrish *et al.* (2000 *Molecular Cell* 6:1077-1087) "for the reasons of record in the Office Action mailed 02/12/2007 and extended to the claims as modified in claim 1."

As set forth in the previous Response filed March 13, 2008, the Examiner alleges that Tuschl *et al.* teach "a siRNA, 19-25 nucleotides in length (see page 4, lines 1-4) wherein the siRNA comprise sugar or backbone modifications to increase in vivo stability and teach a preferred embodiment wherein the 2'-OH group is modified with a 2'-fluoro group, for example (see page 6, lines 3-6)." The Examiner acknowledges that Tuschl *et al.* "does not explicitly teach cytidine or uridine nucleotides in the antisense or sense strands having 2'-fluoro modifications nor specifically teach adenosine or guanosine nucleotides in the antisense or sense strands having 2' modifications."

The Examiner further relies on Eckstein *et al.* for allegedly teaching that "modification of 2' hydroxyl position of the ribose sugar enhances the stability of RNA molecules" and for teaching "preferred modifications of the cytidine and uridine with 2'-fluoro analogues (see column 4, lines 9-25)." The Examiner further relies on Parrish et al. for allegedly teaching that "different chemical modifiers at the 2' position enhance the molecules specificity" and for teaching "modification of the cytidine and uridine nucleotides with a 2' fluoro group as well ds RNA with either the sense or antisense strands unmodified are capable of RNA interference (see Figures 5 and 6)." The Examiner alleges that

> [i]t would have been obvious... to use the general conditions taught by Tuschl *et al.* for making 2'-modified siRNA to discover the optimal number and placement of 2'-sugar modifications in any siRNA molecule, such that the resulting siRNA molecule was endowed with maximum stability and functionality. Additionally, it would have been obvious...to incorporate known modifications, such as 2'-fluoro modifications of cytidine and uridine as taught by Eckstein *et al.* and Parrish *et al.*, to impart increased stability and functionality in any siRNA because as stated by Eckstein *et al.*... RNA has very low stability under physiological conditions and therefore modifications of RNA will provide therapeutic RNA with enhanced stability against chemical and enzymatic degradation.

The Examiner concludes that "the invention as a whole would have been prima facie obvious to one of skill in the art at the time the invention was made."

The test for *prima facie* obviousness is consistent with the legal principles enunciated in *KSR Int'l Co. v. Teleflex Inc.,* 127 S. Ct. 1727 (2007). *Takeda Chem. Indus., Ltd. v. Alpharma Pty., Ltd.,* 2007 U.S. App. LEXIS 15349, at *13 (Fed. Cir. 2007). "While the *KSR* Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test, the Court

acknowledged the importance of identifying 'a *reason* that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does' in an obviousness determination." *Id.* at *13-14 (quoting *KSR,* 127 S. Ct. at 1731) (emphasis added). Although the TSM test should not be applied in a rigid manner, it can provide helpful insight to an obviousness inquiry. *KSR,* 127 S. Ct. at 1731. The *KSR* Court upheld the secondary considerations of non-obviounsess, noting that there is "no necessary inconsistency between the idea underlying the TSM test and the *Graham* analysis." *Id.*

Applicants respectfully traverse this rejection at least for the reasons set forth in Applicants' response filed March 13, 2008, which are reiterated below. Applicants maintain that the cited references, alone and in combination, fail to teach or suggest each and every element of the present invention as recited in the claims amended herein.

Claim 1 and claims 3-4, 33, 39, 84, 85 and 88-90 as they depend from claim 1

Claim 1 (and claims 3-4, 33, 39, 84, 85 and 88-90, as they depend from claim 1) is directed to a small interfering RNA (siRNA), comprising a sense strand and an antisense strand, wherein the antisense strand is complementary to the sense strand and has a sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi), and wherein the ***antisense strand*** is modified by the substitution of ***each*** uridine with a 2'-fluoro uridine and ***each*** cytidine with a 2'-fluoro cytidine, such that *in vivo* stability is enhanced as compared to a corresponding unmodified siRNA, and wherein the siRNA ***retains the ability to inhibit expression of the target mRNA by at least 30%.*** Claim 84 specifies that the sense strand is unmodified, and claim 85 specifies that the sense strand, in addition to the antisense strand, is modified by the substitution of ***each*** uridine with a 2'-fluoro uridine and ***each*** cytidine with a 2'-fluoro cytidine.

The teachings of Tuschl *et al.* are directed to siRNA molecules useful for mediating RNA interference. Tuschl *et al.* teach that "the RNA molecule may contain at least one modified nucleotide analogue," such as "sugar- or backbone-modified ribonucleotides" (page 5, lines 16 and 23-24). Tuschl *et al.* teach that "[i]n preferred sugar-modified ribonucleotides the 2' OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH2, NHR, NR2 or CN, wherein... halo is F, Cl, Br or I" (page 5, line 31 through page 6, line 2). As acknowledged by the Examiner, Tuschl *et al.* fail to teach or suggest the specific sugar-modified

ribonucleotides of ***2'-fluoro uridine and 2'-fluoro cytidine.*** Tuschl *et al.* fail to teach or suggest the combination of these two modified ribonucleotides in a single siRNA. Moreover, Tuschl *et al.* also fail to teach or suggest the ***extent*** and the ***position*** of 2'-fluoro pyrimidines in an siRNA. Specifically, Tuschl *et al.* fail to teach or suggest the substitution of ***each pyrimidine*** of the antisense strand (or of both the antisense and sense strands) with a corresponding 2'-fluoro pyrimidine, as required by the claims. Importantly, Tuschl *et al.* fail to teach or suggest siRNAs comprising 2'-fluoro pyridimidines at ***internal positions***. Indeed, Tuschl *et al.* teach away from the claimed siRNAs comprising internal 2'-fluoro pyrimidines. Specifically, Tuschl *et al.* teach that

> [t]he ***nucleotide analogues may be located at positions where the target-specific activity***, e.g., ***the RNAi mediating activity, is not substantially effected,*** *e.g.*, in a region ***at the 5'-end and/or 3'end of the double-stranded RNA molecule.***"
>
> (see page 5, lines 16-20).

Thus, Tuschl *et al.* ***explicitly teach that nucleotide analogues, e.g., 2'-fluoro modified ribonucleotides, are preferably positioned at the 5'- and/or 3'- ends of the double-stranded RNA molecule***, since internal modifications may affect RNAi mediating activity.

In the previous response filed March 17, 2008, Applicants noted that Tuschl *et al.* teach that "not all positions of a siRNA contribute equally to target recognition" and that "mismatches in the center of the siRNA duplex are most critical and essentially abolish target RNA cleavage." The Examiner states in the Office Action that "Applicants appear to misinterpret the statement by Tuschl *et al.* regarding modifications in the center of the siRNA duplex." The Examiner continues that "Tuschl et al. states *mismatches* i.e. nucleotides that are not complementary to the target strand, in the center of the duplex appear to abolish target RNA cleavage," and that "Tuschl et al. does not make the statement that a chemical modifications of internal positions in the duplex, as instantly claimed, abolish RNAi activity." Applicants respectfully wish to clarify for the record that the foregoing statements by Tuschl regarding *mismatches* and asserting that "not all positions of a siRNA contribute equally to target recogniziton" were not misunderstood by Applicants, but rather were highlighted as being *consistent* with the teachings of Tuschl set forth above with respect to the preferred location of *nucleotide analogs, including 2'-fluoro modified ribonucleotides,* at the 5'- and 3'-ends of the double stranded RNA.

Applicants maintain that one of ordinary skill in the art would not have been motivated, nor have had any reasonable expectation of success, in substituting 2'-fluoro pyrimidines at

internal positions of the antisense strand, let alone at ***each uridine*** and ***each cytidine*** of the antisense strand (or of both the antisense and sense strands), and arrive at an siRNA that ***retains the ability to inhibit expression of the target mRNA by at least 30%***, based on the foregoing teachings of Tuschl *et al.*, since Tuschl *et al.* teach that internal modifications may affect RNAi activity.

The teachings of Eckstein *et al.* fail to make up for the deficiencies of Tuschl *et al.* The teachings of Eckstein *et al.* are generally directed to ***ribozymes*** having enhanced stability against chemical and enzymatical degradation by the incorporation of at least one modified nucleoside. In particular, Eckstein *et al.* teach that a modified nucleoside is one in which "the hydroxyl group at the 2'-position of the ribose sugar is replaced by a modifier group selected from halo, sulfyhdryl, azido, amino, mono-substituted amino and disubstituted amino groups" (column 2, lines 60-65). Eckstein *et al.* fail to teach or suggest the incorporation of any modified nucleoside into an ***siRNA molecule***. It follows that Eckstein *et al.* fail to teach or suggest the ***extent*** or ***position*** of 2'-fluoro pyrimidines in an ***siRNA molecule***, let alone the substitution of ***each*** uridine and cytidine of the ***antisense strand*** (or of both the antisense and sense strands) ***of an siRNA*** with a corresponding 2'fluoro pyrimidine, since the teachings of Eckstein are limited to ribozymes.

Further, one of skill in the art would not have been motivated, or have had a reasonable expectation of success, in introducing the modified nucleosides taught by Eckstein *et al.* into an siRNA, since ribozymes and siRNAs are ***completely different molecules having distinct mechanisms of action***. The skilled artisan would have readily understood, at the time the instant application was filed, that the results obtained for introducing a modified nucleotide into a ribozyme cannot be extrapolated to an siRNA molecule with any reasonable expectation of success because the molecules act through different mechanisms and thus have ***different structural requirements for activity***. In particular, the state of the art at the time of filing recognized that a ribozyme is a self-cleaving ***enzyme***. In contrast, RNAi was recognized at the time of filing to involve the assembly of the RNA molecule with protein components to form a nuclease complex, RNAi-Inducing Silencing Complex (RISC), that RISC utilizes an active mechanism to search for the homologous mRNA target and ultimately mediates cleavage and degradation of the mRNA target. Given the distinct mechanism of RNAi as compared to that of a ribozyme, the ordinary skilled artisan would not have had any reasonable expectation of success in making and using the claimed modified siRNAs. The ordinary skilled artisan would

have recognized that specific modified ribonucleosides tolerated in a ribozyme or in an siRNA cannot be used interchangeably because the distinct molecules operate through distinct mechanisms and therefore have different structural requirements for activity. ***It would not have been predictable, based on the teachings of Eckstein et al., that 2'-flouro pyrimidines and, in particular, 2'-fluoro pyrimidines at internal positions, i.e., positions which base pair with nucleotides lining the cleavage site in the target mRNA, would be compatible with RNAi activity.*** Indeed, Eckstein *et al.* teach that

> 2'-fluoro uridine "incorporated at specific positions of a ribozyme prevented cleavage at these positions by RNase A," and that "protection by incorporation of a modified ribose sugar according to the present invention will be rather general and not be restricted to RNases which depend on the presence of the 2'-hydroxyl group" (column 2, lines 55-59).

Accordingly, one of skill in the art would not have had an expectation of success, based on the teachings of Eckstein *et al.*, to introduce 2'-fluoro modified ribonucleosides into an siRNA, since the teachings of Eckstein *et al.* suggest that such modifications could affect the ability of the siRNA to trigger cleavage and subsequent degradation of the target mRNA. Based on the teachings of Eckstein *et al.*, one of skill in the art could not have predicted with any reasonable expectation of success that an siRNA comprising a 2'-fluoro ribonucleoside would be capable of mediating RNAi, let alone an siRNA comprising 2'-fluoro nucleosides at each cytidine and uridine in the antisense strand, or in both strands, as required by the instant claims.

The teachings of Parrish *et al.* also fail to make up for the deficiencies of Tuschl *et al.* Parrish *et al.* teach modification at the 2' position of the nucleotide sugar in siRNAs and, in particular, "2'-fluoro uracil." Parrish *et al.* fail to teach or suggest an siRNA comprising ***2'-fluoro cytidine.*** Moreover, Parish *et al.* fail to teach or suggest the ***extent*** and the ***location*** of 2'-fluoro pyrimidines in an siRNA, let alone the substitution of ***each*** pyrimidine of the antisense strand (or of both the antisense and sense strands) with a corresponding 2'-fluoro pyrimidine, as required by the instant claims.

In summary, the Examiner has failed to point to any teaching in the Tuschl *et al.*, Eckstein *et al.* and Parrish *et al.* references that would compel one of ordinary skill in the art to make the claimed invention with any reasonable expectation of success. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and [b]oth the suggestion and the ***reasonable***

expectation of success must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Claims 86 and 87

Claim 86, as currently amended, is directed to a small interfering RNA (siRNA), comprising a sense strand and an antisense strand, wherein the antisense strand is complementary to the sense strand and has a sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi), wherein the antisense strand (or the antisense and sense strands) is modified by the substitution of each uridine with a 2'-fluoro uridine and each cytidine with a 2'-fluoro cytidine, wherein the siRNA further comprises a cleavage site for RISC, wherein the antisense strand is further modified by the substitution of ***each adenosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy adenosine and the substitution of each guanosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy guanosine***, and ***wherein the antisense strand comprises at least one 2'deoxy adenosine or 2'-deoxy guanosine,*** such that *in vivo* stability is enhanced as compared to a corresponding unmodified siRNA, and wherein the siRNA retains the ability to inhibit expression of the target mRNA by at least 30%. Claim 87 specifies that the antisense strand is modified by the substitution of ***each adenosine*** with a 2'-deoxy adenosine and the substitution of ***each guanosine*** with a 2'-deoxy guanosine.

As discussed above, Tuschl *et al.* teach siRNAs comprising sugar-modified ribonucleotides, where "the 2' OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH2, NHR, NR2 or CN, wherein... halo is F, Cl, Br or I" (page 5, line 31 through page 6, line 2). As acknowledged by the Examiner, Tuschl *et al.* fail to teach the specific modified ribonucleotides of ***2'-fluoro uridine, 2'-fluoro cytidine, 2'-deoxy guanosine*** and ***2'-deoxy adenosine,*** let alone a ***combination of the foregoing modified ribonucleotides*** in a single siRNA, as required by the claims. Tuschl *et al.* also fail to teach or suggest the ***extent*** and the ***position*** of these modified ribonucleotides in an siRNA. Specifically, Tuschl *et al.* fail to teach or suggest an siRNA in which ***each uridine and each cytidine*** of the antisense strand (or in both the antisense and sense strands) is substituted with a corresponding 2'-fluoro pyrimidine, and in

which ***each guanosine and each adenosine*** of the antisense strand located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand (i.e., at internal positions) is substituted with a corresponding 2'deoxy purine.

Moreover, as discussed above with respect to claim 1, Tuschl *et al.* fail to teach or suggest incorporating any modified nucleotides at an internal position of an siRNA. Indeed, Tuschl *et al.* teach away from an siRNA comprising the specifically claimed combination of internal 2'-fluoro pyrimidines and 2'-deoxy purines. As discussed above, Tuschl *et al.* teach that "the ***nucleotide analogues may be located at positions where the target-specific activity***, e.g., ***the RNAi mediating activity, is not substantially effected,*** *e.g.*, in a region ***at the 5'-end and/or 3'end of the double-stranded RNA molecule"*** (see page 5, lines 16-20). Thus, Tuschl *et al.* ***explicitly teach that nucleotide analogues, e.g., 2'-deoxy ribonucleotides, are preferably positioned at the 5'- and/or 3'-ends of the double-stranded RNA molecule***, since internal modifications may affect RNAi mediating activity. The ordinary skilled artisan would not have had the motivation or a reasonable expectation of success, based on the teachings of Tuschl *et al.*, to introduce the specific combination of 2'-fluoro pyrimidines and 2'-deoxy purines at ***internal*** positions of an siRNA antisense strand, let alone to the extent and at the particular positions required by the claims (i.e., substitution of ***each*** pyrimidine of the antisense strand, or of both the antisense and sense strands, with a corresponding 2'-fluoro pyrimidine, combined with the substitution of ***each*** purine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a corresponding 2'-deoxy purines, or the substitution of ***each*** purine of the antisense strand with a corresponding 2'-deoxy purine), since Tuschl *et al.* teach that internal modifications may affect RNAi activity.

Indeed, Applicants' own specification teaches that replacing either the antisense strand or both strands entirely with 2'-deoxynucleotides ***completely blocked siRNA function*** (see, *e.g.*, page 88, lines 24-25 of the specification and Figure 13B, lanes 7-15). Applicants respectfully submit to the Examiner that the siRNAs of claims 86-87 are based on Applicants' ***unexpected result*** that a ***combination of 2'-fluoro pyrimidines and 2'-deoxy purines could rescue siRNA function that was lost due to incorporation of 2'-deoxynucleotides in the antisense strand***. Specifically, Applicants teach that

> [i]n general, mixing 2'-Fluro modification with deoxy modification could rescue siRNA function (Figure 13B, lanes 25-60). When ***2' FU, FC*** nucleotides were incorporated into the EGFP siRNA ***antisense strand*** with ***guanine and adenine deoxynucleotides at positions 9, 10 and 13,*** which base pair with nucleotides

> lining the cleavage site, (Figure 13A), EGFP RNAi effects were ***almost indistinguishable from wild type levels*** (Figure 13B, lanes 25-33; Table 1, row 5). In addition, siRNAs that had the entire antisense strand replaced with 2' FU, 2' FC, dATP and dGTP nucleotides still showed moderate levels of RNAi activity ~ 42%, or ~44% if the sense strand was also modified with 2' FU, FC (figure 13B, lanes 52-60; Table 1, rows 7, 8). (See page 88, lines 24-30 of the specification.)

Thus, the ordinary skilled artisan could not possibly have predicted with any expectation of success, prior to Applicants' invention, that an siRNA comprising the specific combination of modifications, *e.g.*, 2'-fluoro pyrimidines and 2'-deoxy purines, to the extent and at the specific positions required by the instant claims, would surprisingly retain the ability to mediate RNAi, let alone be capable of inhibiting expression of the target mRNA by at least 30%.

The teachings of Eckstein *et al.* fail to make up for the deficiencies of Tuschl *et al.* As discussed above with respect to claim 1, the teachings of Eckstein *et al.* are directed to ***ribozymes*** having enhanced stability against chemical and enzymatical degradation by the incorporation of at least one modified nucleoside. In particular, Eckstein *et al.* teach that a modified nucleoside is one in which "the hydroxyl group at the 2'-position of the ribose sugar is replaced by a modifier group selected from ***halo, sulfyhdryl, azido, amino***, mono-substituted amino and disubstituted amino groups" (column 2, lines 60-65). Eckstein *et al.* fail to teach or suggest the incorporation of ***any*** modified nucleoside into an ***siRNA molecule***. The Eckstein *reference* is also ***devoid of any teaching regarding the incorporation of 2'-deoxy modified ribonucleotides*** in any molecule for enhanced stability. Accordingly, Eckstein *et al.* fail to teach or suggest an siRNA comprising the specific sugar-modified ribonucleotides of ***2'-deoxyguanosine and 2'-deoxyadenosine,*** let alone the ***specific combination*** of 2'-deoxy purines and 2'-fluoro pyrimidines present to the ***extent*** and at the ***positions*** required by claims 86-87.

Moreover, Eckstein et al. ***teach away*** from the incorporation of 2'-deoxynucleosides into siRNA molecules. In the single instance at which Eckstein *et al.* refer to 2'-deoxynucleotides, Eckstein *et al.* report that "the incorporation of only 15% 2'-deoxynucleotides into a hammerhead ribozyme is reported to decrease the catalytic efficiency by two orders of magnitude" (column 7, lines 13-17 and 22-25). Thus, the ordinary skilled artisan would not have had the motivation or a reasonable expectation of success, based upon the teachings of Eckstein *et al.*, to incorporate 2'-deoxynucleosides at any position in an siRNA, since Eckstein *et al.* teach that 2'-deoxynucleosides decreased activity of an RNA molecule into which they were introduced.

Further, as set forth above with respect to claim 1, one of skill in the art would not have had a reasonable expectation of success in introducing any modified nucleosides into an siRNA based on Eckstein *et al.*, since ribozymes and siRNAs are ***completely different molecules having distinct mechanisms of action***. The skilled artisan would have readily understood, at the time the instant application was filed, that the results obtained for introducing a modified nucleotide into a ribozyme cannot be extrapolated to an siRNA molecule with any reasonable expectation of success because the molecules act through different mechanisms and thus have ***different structural requirements for activity***. In particular, the state of the art at the time of filing recognized that a ribozyme is a self-cleaving ***enzyme***. In contrast, RNAi was recognized at the time of filing to involve the assembly of the RNA molecule with protein components to form a nuclease complex, RNAi-Inducing Silencing Complex (RISC), that RISC utilizes an active mechanism to search for the homologous mRNA target and ultimately mediates cleavage and degradation of the mRNA target. Given the distinct mechanism of RNAi as compared to that of a ribozyme, the ordinary skilled artisan would not have had any reasonable expectation of success in making and using the claimed modified siRNAs. The ordinary skilled artisan would have recognized that specific modified ribonucleosides tolerated in a ribozyme or in an siRNA cannot be used interchangeably because the distinct molecules operate through distinct mechanisms and therefore have different structural requirements for activity. ***It would not have been predictable, based on the teachings of Eckstein et al., that 2'-flouro pyrimidines and 2'-deoxy purines at internal positions, i.e., positions which base pair with nucleotides lining the cleavage site in the target mRNA, would be compatible with RNAi activity.*** Indeed, Eckstein *et al.* teach that 2'-fluoro uridine "incorporated at specific positions of a ribozyme prevented cleavage at these positions by RNase A," and that "protection by incorporation of a modified ribose sugar according to the present invention will be rather general and not be restricted to RNases which depend on the presence of the 2'-hydroxyl group" (column 2, lines 55-59). Accordingly, one of skill in the art would not have had an expectation of success, based on the teachings of Eckstein *et al.*, to introduce 2'-fluoro modified ribonucleosides and 2'-deoxy modified ribonucleosides into an siRNA, since the teachings of Eckstein *et al.* suggest that at least 2'-fluoro modifications could affect the ability of the siRNA to trigger cleavage and subsequent degradation of the target mRNA.

The teachings of Parrish *et al.* also fail to make up for the deficiencies of Tuschl *et al.* As discussed above, Parrish *et al.* teach modification at the 2' position of the nucleotide sugar in siRNAs, including 2'-fluoro uracil, 2'-deoxy thymidine and 2'-deoxy cytidine. Parrish *et al.* fail to teach or suggest the specific sugar-modified ribonucleotides of ***2'-fluoro cytidine, 2'-deoxy guanosine and 2'-deoxy adenosine,*** let alone the specific combination of the foregoing modified ribonucleotides together with 2'-fluoro uridine in a single siRNA. Parrish *et al.* also fail to teach or suggest the ***extent*** and the ***position*** of the foregoing modified ribonucleotides in an siRNA, as required by the instant claims.

Moreover, Parrish et al. ***teach away*** from the claimed invention of claims 86-87. In particular, Parrish *et al.* teach that "[m]odification of cytidine to deoxycytidine (or uracil to thymidine) on either the sense or the antisense strand of the trigger was sufficient to produce a ***substantial decrease in interference activity***" and that, for this modification, "***trigger activity was more sensitive to modification of the antisense strand*** than that of the sense strand." Thus, Parrish *et al.* teach that 2'-deoxy nucleosides are not well tolerated in siRNAs, and, in particular, are not well tolerated in the antisense strand. Accordingly, one of ordinary skill in the art would not have had the motivation, or a reasonable expectation of success, based on the teachings of Parrish *et al.*, to substitute any ribonucleoside of the ***antisense strand*** of an siRNA with a corresponding ***2'-deoxy nucleoside*** (*e.g.*, either at internal positions or throughout the entire antisense strand), since Parrish *et al.* teach that siRNAs containing 2'-deoxy nucleosides in the antisense strand have substantially decreased interference activity.

In summary, the Examiner has failed to point to any teaching in the Tuschl *et al.*, Eckstein *et al.* and Parrish *et al.* references that would compel one of ordinary skill in the art to make the claimed invention with any reasonable expectation of success. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and [b]oth the suggestion and the ***reasonable expectation of success*** must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

In view of the foregoing, Applicants respectfully request that the rejection of claims 1, 3-4, 33, 38 and 84-90 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* in view of Eckstein *et al.* and Parrish *et al.* be reconsidered and withdrawn.

Rejection of Claims 1 and 86 under 35 USC § 103(a)

The Examiner has rejected claims 1 and 86 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* (WO 22/44321) in view of Eckstein *et al.* (US 5,672,695), Parrish *et al.* (2000 *Molecular Cell* 6:1077-1087) and Allerson *et al.* (US 2005/0026160). The Examiner relies on Tuschl *et al.*, Eckstein *et al.* and Parrish *et al.* for the reasons set forth above. The Examiner further relies on Allerson *et al.* for allegedly teaching "siRNA comprising 2'-fluoro groups as well as 2'-deoxynucleotides wherein the modified nucleotides are on the sense or antisense strand" and that "said ***siRNA are capable of eliciting RNAi in cells (see Examples 1-7)***." The Examiner further relies on Allerson et al. for teaching "said modified siRNA were ***capable of retaining activity up 86%*** compared to untreated controls (see for example paragraph 0365)."

With respect to claim 86, the Examiner notes that the claim "is given its reasonable broadest interpretation" and "because there is no specific sequence claimed, it is conceivable that a siRNA would not have an adenosine or guanosine located in the region of the cleavage site on the antisense strand and therefore the siRNA would not have any substitutions with a 2'-deoxy adenosine or guanosine." The Examiner continues that "[g]iven this breadth of possibilities of substitution of an adenosine or guanosine in the claimed siRNA, and given the different modifications shown by Parrish *et al.* and Allerson *et al.*, one of skill in the art would have been motivated to search for particular chemical modifications by routine experimentation to determine the optimum number and placement of the 2'-fluoro and deoxynucleotide modifications in either the sense or antisense strand of a dsRNA that would enhance the molecules stability." The Examiner concludes that "the invention as a whole would have been prima facie obvious to one of skill in the art at the time the invention was made."

The test for *prima facie* obviousness is consistent with the legal principles enunciated in *KSR Int'l Co. v. Teleflex Inc.,* 127 S. Ct. 1727 (2007). *Takeda Chem. Indus., Ltd. v. Alpharma Pty., Ltd.,* 2007 U.S. App. LEXIS 15349, at *13 (Fed. Cir. 2007). "While the *KSR* Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test, the Court

acknowledged the importance of identifying 'a *reason* that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does' in an obviousness determination." *Id.* at *13-14 (quoting *KSR,* 127 S. Ct. at 1731) (emphasis added). Although the TSM test should not be applied in a rigid manner, it can provide helpful insight to an obviousness inquiry. *KSR,* 127 S. Ct. at 1731. The *KSR* Court upheld the secondary considerations of non-obviounsess, noting that there is "no necessary inconsistency between the idea underlying the TSM test and the *Graham* analysis." *Id.*

Applicants respectfully traverse this rejection. Applicants submit that the primary reference Tuschl *et al.*, in combination with the secondary references Eckstein *et al.* and Parrish *et al.,* fail to render obvious the siRNAs of claims 1 and 86, for at least the reasons set forth above. Applicants further submit that the additional secondary reference, Allerson *et al.*, fails to make up for the deficiencies of the foregoing references.

As an initial matter, Applicants respectfully submit that Allerson *et al.* was filed on November 4, 2003, a filing date which is subsequent to Applicants' filing date of September 25, 2003, as well as the filing dates of each of Applicants' four priority documents. Accordingly, the disclosure of Allerson *et al.* that is allegedly available as prior art against Applicants' claims is limited to the disclosure contained in the Allerson *et al.* priority document, U.S. Serial No. 60/423,760 (hereinafter referred to as "the '760 application"), filed November 5, 2002.

Claim 1

The teachings of the '760 application are limited to a general disclosure regarding the incorporation of modified nucleosides, *e.g.*, 2'-fluoro nucleosides, into siRNAs. Applicants respectfully submit that Examples 1-7, to which the Examiner specifically refers in the Office Action, are not present in the '760 application. Indeed, Applicants respectfully submit that the '760 application is devoid of any data showing that modified siRNAs were actually made, let alone data showing that said siRNAs are "capable of eliciting RNAi in cells" or " capable of retaining activity up 86% compared to untreated controls," as asserted by the Examiner. The '760 application merely describe prophetic "representative siRNA oligomers" (see page 37 of the '760 application), none of which correspond to the siRNAs described in Examples 2 and 3 of Allerson *et al.*. Accordingly, the '760 application fails to teach or suggest that the claimed modified siRNAs, *e.g.*, comprising substitution of each uridine and each cytidine of the antisense

strand (or of both the antisense and sense strands) with 2'-fluoro uridine and 2'-fluoro cytidine, are able to inhibit expression of a target mRNA by at least 30%, as required by the claims.

Claim 86

Claim 86, as currently amended, is directed to a small interfering RNA (siRNA), comprising a sense strand and an antisense strand, wherein the antisense strand is complementary to the sense strand and has a sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi), wherein the antisense strand (or the antisense and sense strands) is modified by the substitution of each uridine with a 2'-fluoro uridine and each cytidine with a 2'-fluoro cytidine, wherein the siRNA further comprises a cleavage site for RISC, wherein the antisense strand is further modified by the substitution of each adenosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy adenosine and the substitution of each guanosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy guanosine, and ***wherein the antisense strand comprises at least one 2'deoxy adenosine or 2'-deoxy guanosine,*** such that *in vivo* stability is enhanced as compared to a corresponding unmodified siRNA, and wherein the siRNA retains the ability to inhibit expression of the target mRNA by at least 30%.

With respect to claim 86, Applicants submit that the teaching of the '760 application regarding the incorporation of 2'-deoxy nucleosides into an siRNA is limited to two single prophetic "representive siRNA oligomers" (see page 37, lines 33-34) containing 2'-deoxy uridine, cytidine and guanosine. The '760 application fails to teach or suggest the specific 2'-deoxy ribonucleoside of ***2'-deoxy adenosine***. The '760 application does not describe the actual making of said siRNAs, and thus also fails to teach or suggest that said siRNAs are able to mediate RNAi, let alone that they are able to inhibit expression of a target mRNA by at least 30%, as required by the claims.

Moreover, Applicants submit that the '760 application ***teaches away*** from the claimed invention. For example, the '760 application teaches that the "substitution of the 4 nucleosides from the 3'-end with 2'-deoxynucleosides has been demonstrated to not affect activity... [while] [o]n the other hand, ***substitution with 2'deoxynucleosides... throughout the sequence (sense or antisense) was shown to be deleterious to RNAi activity***" (see page 5, lines 25-28, of the '760

application). The '760 application further teaches that "RNA-DNA heteroduplexes did not serve as triggers for RNAi" (page 6, line 11 of the '760 application). Based on the foregoing disclosure of the '760 application, the ordinary skilled artisan would not have been motivated, or have had any reasonable expectation of success, in producing siRNAs containing ***2'-deoxy nucleosides at internal positions*** of the antisense strand of an siRNA, let alone containing the claimed combination of 2'fluoro pyrimidines throughout the antisense strand and 2'-deoxy purines at internal positions of the antisense strand, and that retain the ability to inhibit expression of a target mRNA by at least 30%, since the '760 application teaches that substitution with 2'-deoxy nucleosides throughout the antisense sequence, *e.g.*, at internal positions, was "deleterious to RNAi activity."

Indeed, as discussed above, Applicants' own specification teaches that replacing either the antisense strand or both strands entirely with 2'-deoxynucleotides ***completely blocked siRNA function*** (see, *e.g.*, page 88, lines 24-25 of the specification and Figure 13B, lanes 7-15). Applicants submit that the siRNAs encompassed by claim 86 are based on the ***unexpected result*** that a ***combination of 2'-fluoro pyrimidines and 2'-deoxy purines could rescue siRNA function that was lost due to incorporation of 2'-deoxynucleotides in the antisense strand***. Specifically, Applicants teach that

> [i]n general, mixing 2'-Fluro modification with deoxy modification could rescue siRNA function (Figure 13B, lanes 25-60). When ***2' FU, FC*** nucleotides were incorporated into the EGFP siRNA ***antisense strand*** with ***guanine and adenine deoxynucleotides at positions 9, 10 and 13,*** which base pair with nucleotides lining the cleavage site, (Figure 13A), EGFP RNAi effects were ***almost indistinguishable from wild type levels*** (Figure 13B, lanes 25-33; Table 1, row 5). In addition, siRNAs that had the entire antisense strand replaced with 2' FU, 2' FC, dATP and dGTP nucleotides still showed moderate levels of RNAi activity ~ 42%, or ~44% if the sense strand was also modified with 2' FU, FC (figure 13B, lanes 52-60; Table 1, rows 7, 8).

Thus, the ordinary skilled artisan could not possibly have predicted with any expectation of success, prior to Applicants' invention, that an siRNA comprising a combination of 2'-fluoro pyrimidines and 2'-deoxy purines, present to the extent and at the specific positions required by the instant claims, would surprisingly retain the ability to mediate RNAi, let alone be capable of inhibiting expression of the target mRNA by at least 30%.

In summary, the Examiner has failed to point to any teaching in the Tuschl *et al.*, Eckstein *et al.*, Parrish *et al.* and Allerson *et al.* references that would compel one of ordinary

skill in the art to make the claimed invention with any reasonable expectation of success. The prior art must suggest "to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process" and [b]oth the suggestion and the ***reasonable expectation of success*** must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

In view of the foregoing, Applicants respectfully request that the rejection of claims 1 and 86 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* in view of Eckstein *et al.*, Parrish *et al.* and Allerson *et al.* be reconsidered and withdrawn.

CONCLUSION

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

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Respectfully submitted,

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